Michigan Green Chemistry and Engineering Conference 2012

Development of a cell-free neurochemical screening battery to predict adverse outcomes in mammals, fish, and birds

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Toxicity Testing and Green Chemistry

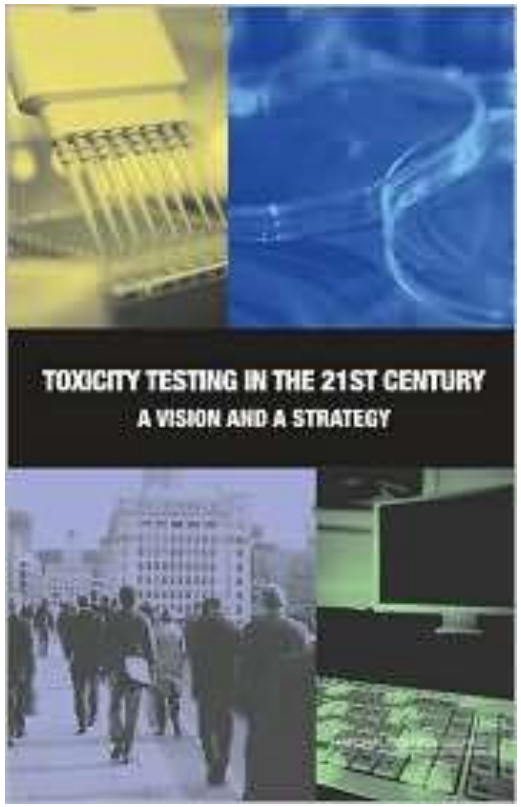
- **Green chemistry:**
  - Is the design of chemical products and processes that reduce or eliminate the use or generation of **hazardous substances** (USEPA)
  - Consists of chemicals and chemical processes designed to **reduce or eliminate negative environmental impacts** (USEPA)
- **Toxicity testing:**
  - Aids in identification of potentially hazardous chemicals
    - Human health
    - Ecological health

Toxicity Testing – Is it broken?

- **Risk Assessment:**
  - Fundamental to public and ecological health
  - Informs decision makers
  - Evolving (tools, knowledge, uncertainty, susceptibilities, etc)

- **Problems and Challenges:**
  - 80,000+ chemicals (+mixtures) untested towards humans
  - 80,000+ chemicals (+mixtures) **untested across 1000s of fish, mammals, birds**
  - Chemical + non-chemical stressor interactions
  - Current testing methods: expensive, slow, low throughput, apical endpoints, limited MOA studies, etc.

NRC Vision and Strategy for Toxicity Testing



VISION

"Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods… use computational models"

STRATEGY

- **Innovate (screening, toxicity pathways, high-throughput, computation, omics)**
- **Reduce use of whole animal studies (RRR) & apical endpoints**

Vision & Strategy:

Predictive Ecotoxicology in the 21st Century

Daniel L. Villeneuve* and Natàlia Garcia-Reyero

In the 20th century, predicting ecological risk from the use of certain chemicals relied on testing programs that directly measured adverse outcomes (death, disease, reproductive failure, or developmental dysfunction) using in vivo toxicity tests. Extrapolation from these tests—from one species to another or from controlled laboratory tests to uncontrolled real-world environments—was based on largely conservative assumptions or arbitrary uncertainty factors. The result? Costly, time-consuming, unfocused, and contentious assessments that often failed to inspire public confidence in related regulatory and policy decisions.

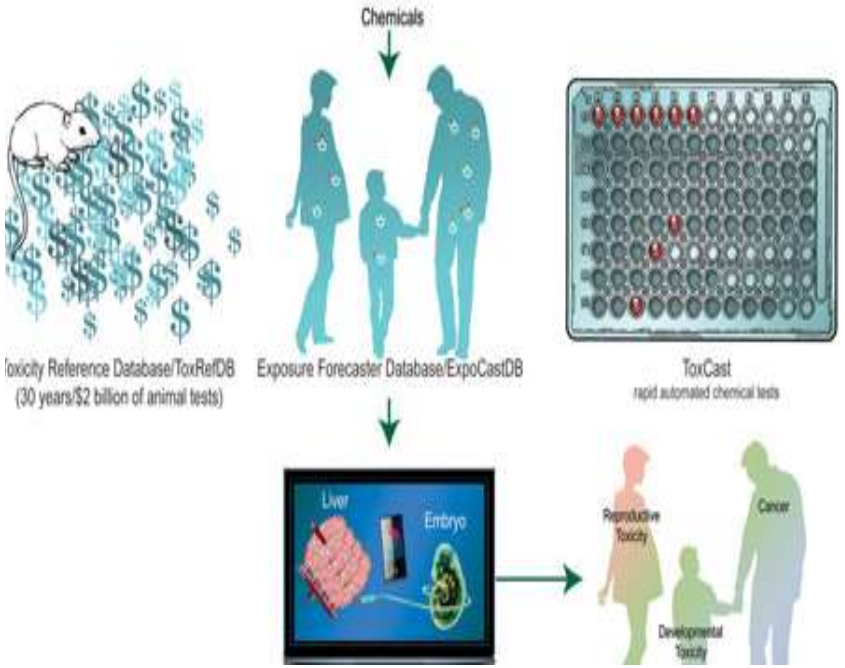
Environmental Toxicology and Chemistry, Vol. 30, No. 1, January 2011

ToxCast™

Screening Chemicals to Predict Toxicity Faster and Better

EPA launched ToxCast in 2007 to develop ways to predict potential toxicity and to develop a cost-effective approach for prioritizing the thousands of chemicals that need toxicity testing.

- Uses advanced science tools to help understand how human body processes are impacted by exposures to chemicals and helps determine which exposures are most likely to lead to adverse health effects.
- Includes over 650 state-of-the-art rapid tests (called high-throughput assays) that are screening 1,000 environmental chemicals for potential toxicity.



http://www.epa.gov/ncct/toxcast/ [last accessed Oct 25, 2012]

In Vitro Screening of Environmental Chemicals for Targeted Testing Prioritization: The ToxCast Project

Richard S. Judson, Keith A. Houck, Robert J. Kavlock, Thomas B. Knudsen, Matthew T. Martin, Holly M. Mortensen, David M. Reif, Daniel M. Rotroff, Imran Shah, Ann M. Richard, and David J. Dix

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Background: Chemical toxicity testing is being transformed by advances in biology and computer modeling, concerns over animal use, and the thousands of environmental chemicals lacking toxicity data. The U.S. Environmental Protection Agency's ToxCast program aims to address these concerns by screening and prioritizing chemicals for potential human toxicity using *in vitro* assays and *in silico* approaches.

Objectives: This project aims to evaluate the use of *in vitro* assays for understanding the types of molecular and pathway perturbations caused by environmental chemicals and to build initial prioritization models of *in vivo* toxicity.

Methods: We tested 309 mostly pesticide active chemicals in 467 assays across nine technologies including hi and cell line genes and pa

Results: Ch saw many ex Chemicals ra We found a by a chemica toxicity. We formation.

Conclusion environmen

Key words: *Health Persp*

changes in biological pathways that are associated with *in vivo* end points and that could potentially lead to human disease. Chemicals whose properties and assay profiles match these predictive signatures can be prioritized for more in-depth testing, which may include nontraditional, mechanism-focused *in vitro* tests. In this article, we provide an overview of the entire ToxCast phase I assay results data

Table 1

Summary of the ToxCast *in vitro* assays: types of cells, number of concentrations (concentration range), time points, and types of readout

Assay set	Assays	Cell type	Concentrations (μM)	Time points	Readout
Cell-free HTS	239	Cell free	CYP assays: 8 (0.00914–20) All others: 8 (0.0229–50)	1	IC_{50}
Cell-based HTS	13	HEK293, HeLa, HepG2, FAO	15 (0.0012–92)	1	IC_{50}
High-content cell imaging	19	HepG2 and primary rat hepatocytes	10 (0.39–200)	3 (1, 24, 72 hr)	IC_{50}
Quantitative Nuclease protection	16	Primary human hepatocytes	5 (0.004–40)	3 (6, 24, 48 hr)	IC_{50}
Multiplex transcription reporter	81	HepG2	7 (0.0014–100)	1	LEC
Biologically multiplexed activity profiling (BioMAP)	87	HUVEC, HDFn, HBEC, ASMC, KC, PBMC	4 (1.48–40)	1	LEC (separate up- and down-regulation readouts)
Phase I and II XME cytotoxicity	4	Hep3B	9 (0.0146–960)	1	IC_{50}
HTS genotoxicity	1	TK6	3 (50–200)	1	LEC
Real-time cell electronic sensing	7	A549	8 (0.047–100)	Continuous (0–48 hr)	IC_{50}, LEC

Abbreviations: A549, human alveolar basal epithelial cell carcinoma cell line 549; ASMC, arterial smooth muscle cells; CYP, cytochrome P450; FAO, Reuber rat hepatoma cell line; HBEC, human bronchial epithelial cells; HDFn, human neonatal foreskin fibroblasts; HEK293, Human embryonic kidney cell line 293; HeLa, Henrietta Lacks cervical cancer cell line; Hep3B, hepatocellular carcinoma cell line 3b; HepG2, hepatocellular carcinoma cell line G2; HUVEC, human umbilical vein endothelial cells; KC, keratinocytes; PBMC, peripheral blood mononuclear cells; TK6, T-cell blast cell line 6. Data were collected in concentration–response format for each chemical–assay pair. If data were fit to a Hill function, we report the AC_{50} values. In other cases, an LEC was determined by significant change relative to negative control. Assay methods are described in more detail in Supplemental Material (doi:10.1289/ehp.0901392).

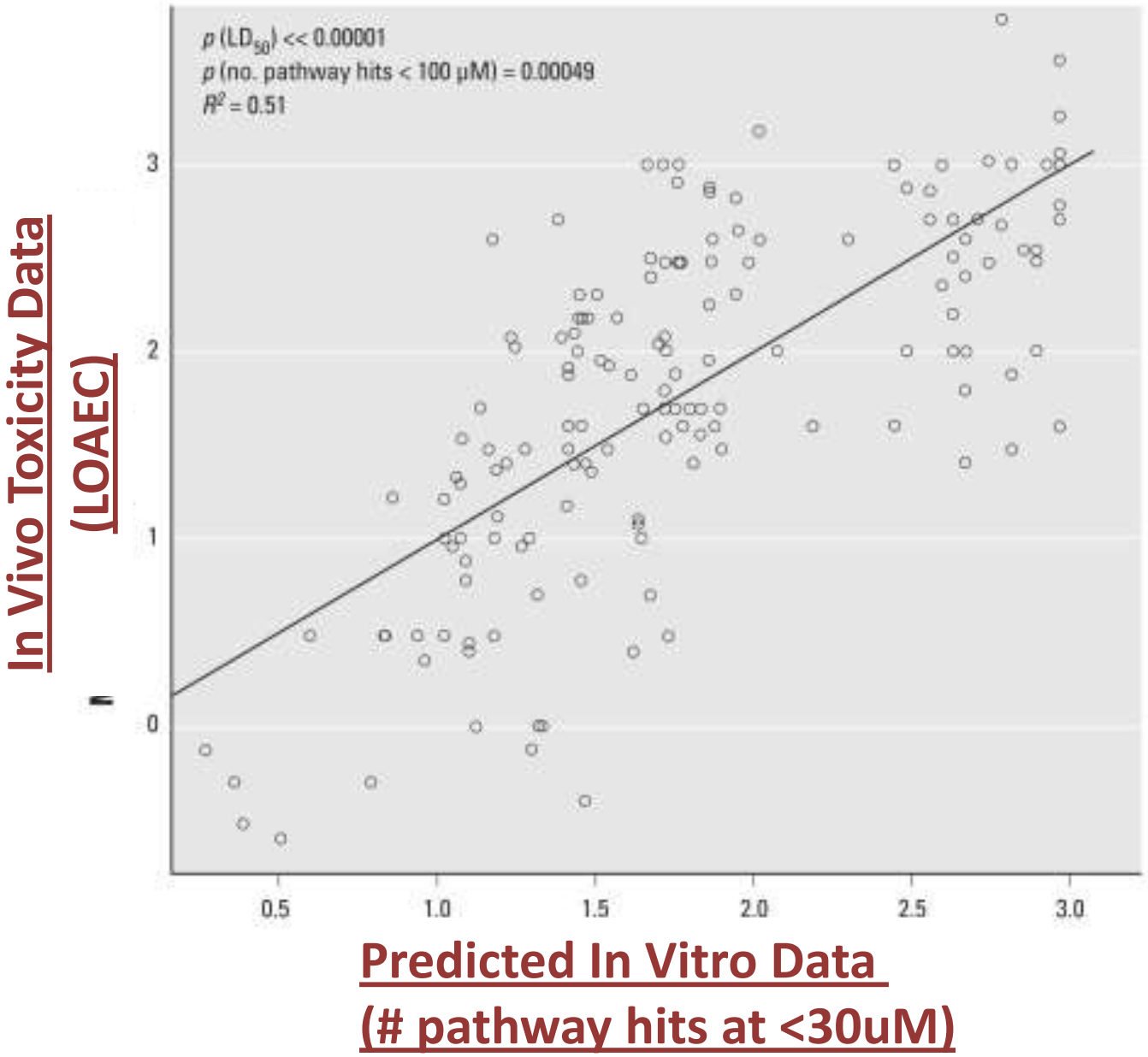


Figure 5

Association between the number of minimal pathway hits (which we assume is inversely correlated with the minimum concentration at which significant pathway activity occurs for the chemical) and the lowest dose *in vivo* at which a significant toxicity end point is observed, in this case for the rat prenatal developmental bioassay. Each point represents a single chemical. The *x*-axis is the value resulting from the fitted model, which is $0.6 + 0.4 \times \log_{10}(LD_{50}) - 0.037 \times$ (number of minimal pathway hits at concentrations < 30 µM). The *y*-axis is the minimum $\log_{10}$(concentration) at which toxicity is seen for this study type. This analysis was performed on the 153 chemicals for which we had all values.

Why Cell Free Systems

- ERA: potentially 1,000s of mammals, fish, birds
  - Limited availability of test organisms (eg. at-risk species, husbandry, biological knowledge, etc.)
  - Societal & monetary concerns over animal bioassays
  - Limited cell-based tools (cell lines, cell cultures)
- Simplified platforms to assess interactions
- Pathway-based high throughput screening assays → quantitative models
- Multiple species, chemicals, pathways
- Cheap, quick, and hypothesis generating
- Component of ToxCast
- Limitations? Over-simplification; single 'Tool'

TODAY: ToxCast → "ECO"ToxCast

OBJECTIVES

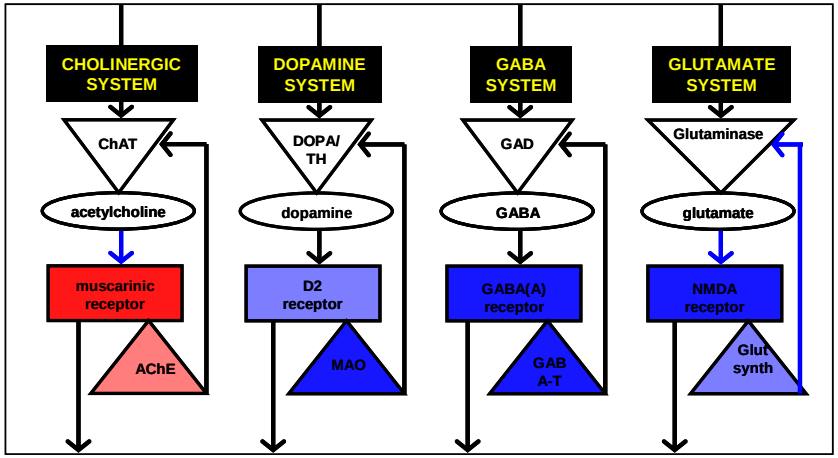
- advance an in vitro, **cell-free** high-throughput neurochemical **screening assay** platform
- **mammals, fish, birds** (+biomedical, humans)
- model data outputs to **predict adverse effects**

HYPOTHESIS

- Several toxicants will emerge that interact with, and disrupt the function of, neurotransmitter receptors, enzymes and transporters that mediate vertebrate reproduction

Guiding Principle Revisited

30,000 pollutants



Neurotoxic

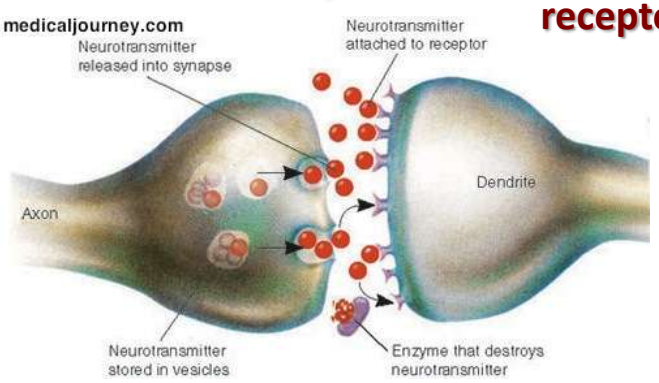
(reproduction; neurobehavior)

How Cell Free Systems

- Obtain brain tissue
- Isolate cellular components
- Perform assay in microplates
- Calculate IC50s & model data outputs

transporters

receptors



enzymes

chemical 1

chemical 2

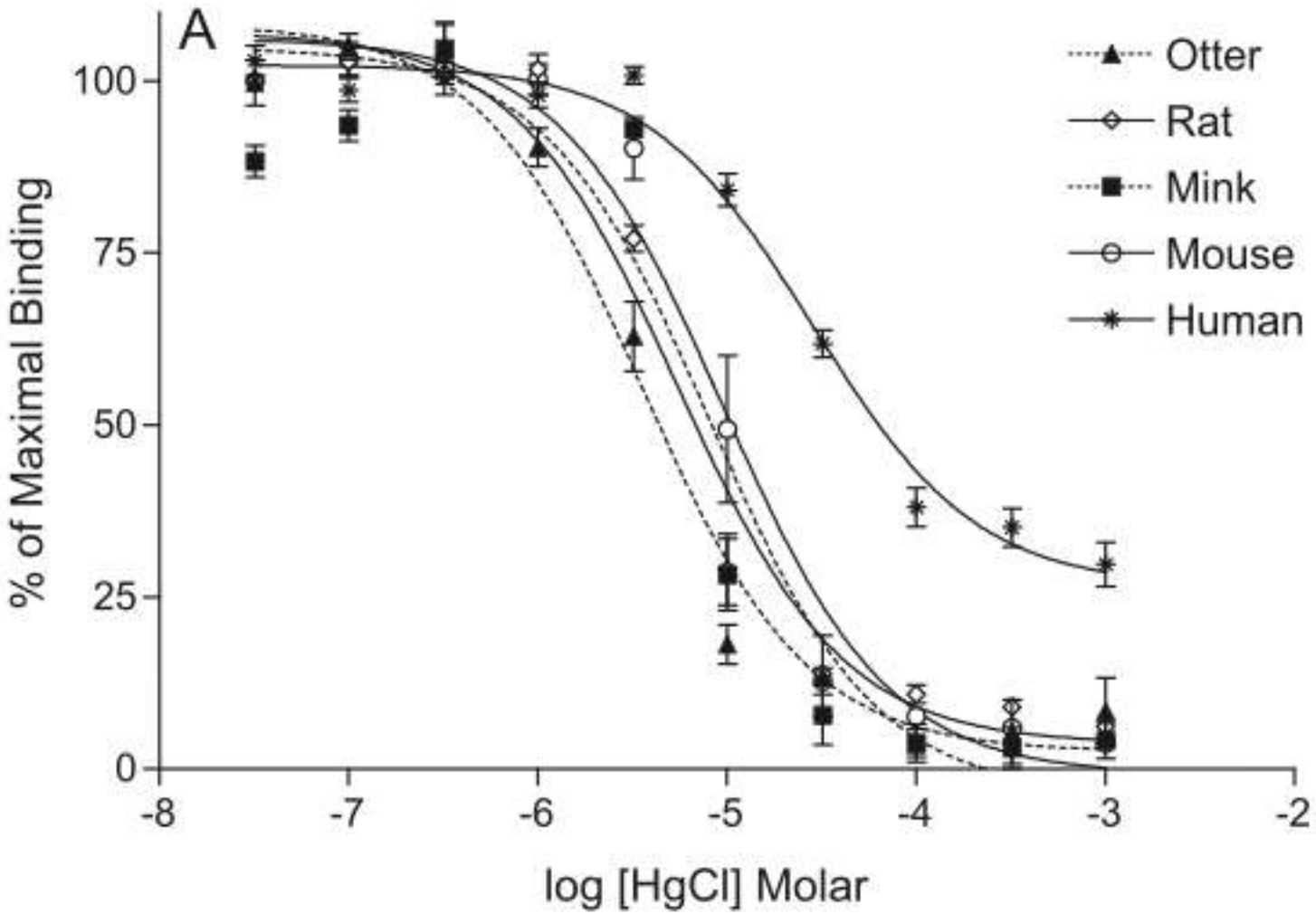
chemical 3

A) Multiple Species (ecological & biomedical)

ELSEVIER
Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology and Applied Pharmacology 205 (2005) 71–76
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

An interspecies comparison of mercury inhibition on muscarinic acetylcholine receptor binding in the cerebral cortex and cerebellum

Niladri Basu[a,b], Christopher J. Stamler[a,c], Kovana Marcel Loua[a], Hing Man Chan[a,c,*]

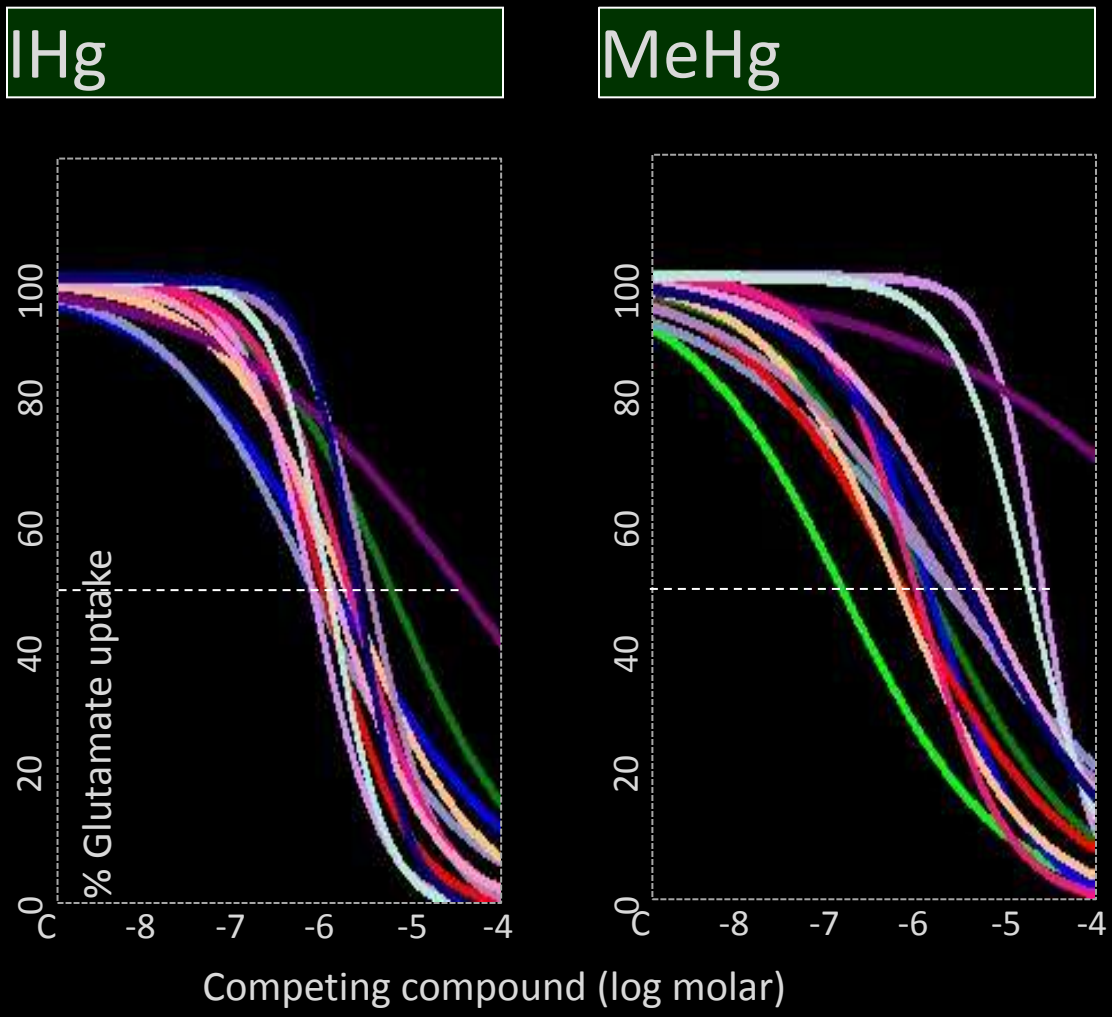


Inhibition of Glutamate Uptake

EC50

(Glu uptake)

Mammal	Porpoise
	Seal
	Mouse
	C dolphin
	Otter
	A dolphin
	Mink
Bird	Eagle
	Chicken
Fish	M shark
	L shark
	Zebrafish
	Seatrout



B) Multiple Toxicants (non-model species)

Journal of Toxicology and Environmental Health, Part A, 69:1133–1143, 2006

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Taylor & Francis

MERCURY BUT NOT ORGANOCHLORINES INHIBITS MUSCARINIC CHOLINERGIC RECEPTOR BINDING IN THE CEREBRUM OF RINGED SEALS (*PHOCA HISPIDA*)

Niladri Basu[1], Michael Kwan[2], Hing Man Chan[3,4]

TABLE 1. Effects of Environmental Neurotoxicants on the Binding of [^{3}H]Quinuclidinyl Benzilate ([^{3}H]-QNB) to the Muscarinic Cholinergic (mACh) Receptor in Cellular Membranes Isolated From the Cerebrum ($n = 8$) of Wild Ringed Seals (*Phoca hispida*)

Environmental neurotoxicant	mACh receptor binding (% of control)
Hg^{2+}	2.89 ± 0.63[a]
$MeHg^{+}$	21.52 ± 0.65[a]
DDT	94.13 ± 6.40
Dieldrin	91.92 ± 4.94
Chlordane	105.01 ± 3.90
Lindane	103.26 ± 7.50
Arochlor 1254	102.96 ± 5.27
Toxaphene	77.58 ± 2.84[a]

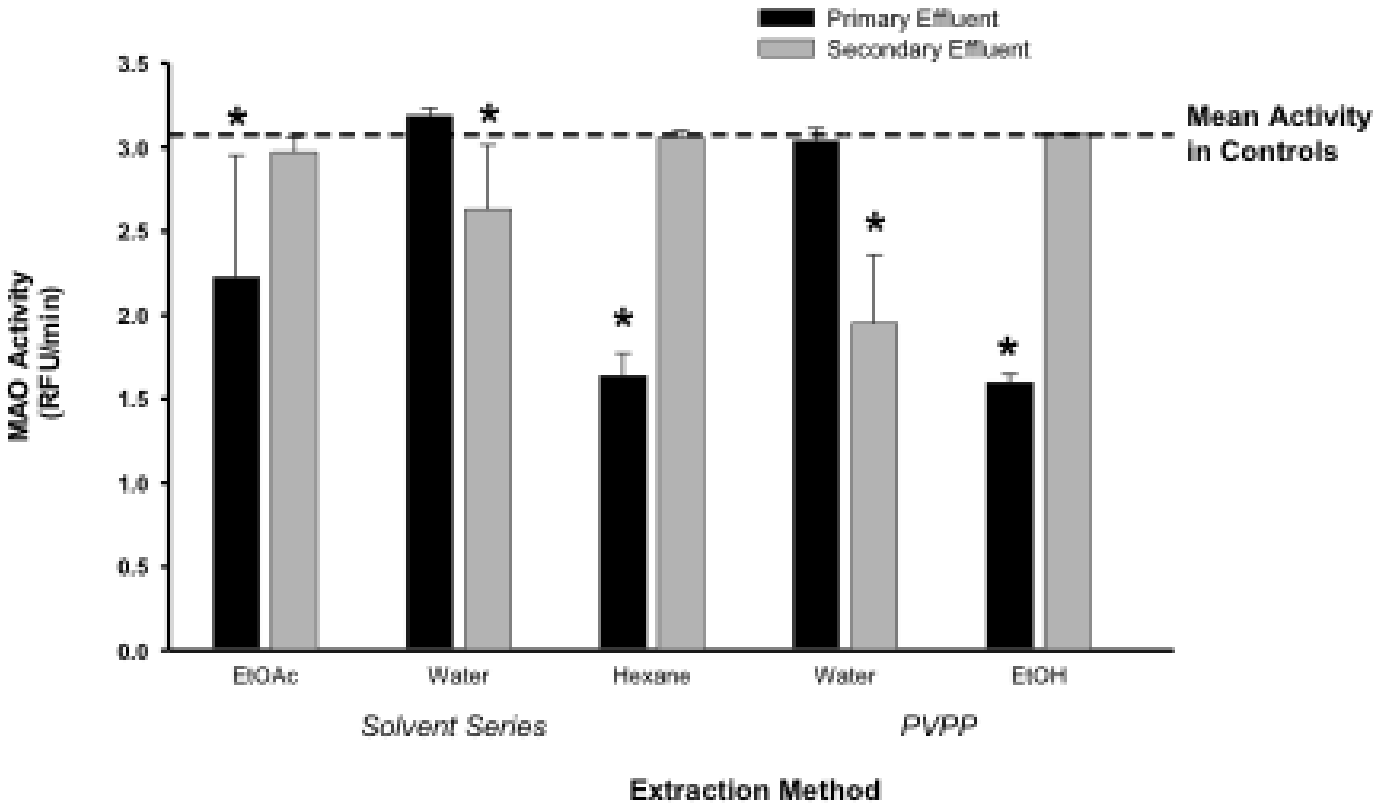
Note. Data represent mACh receptor binding in the presence of the highest concentration of neurotoxicant tested (320 μ*M*) as a percent of binding in nonexposed samples.

[a]Statistical differences ($p < .05$) between exposed and nonexposed samples.

C) Multiple Assays (ecological applications)

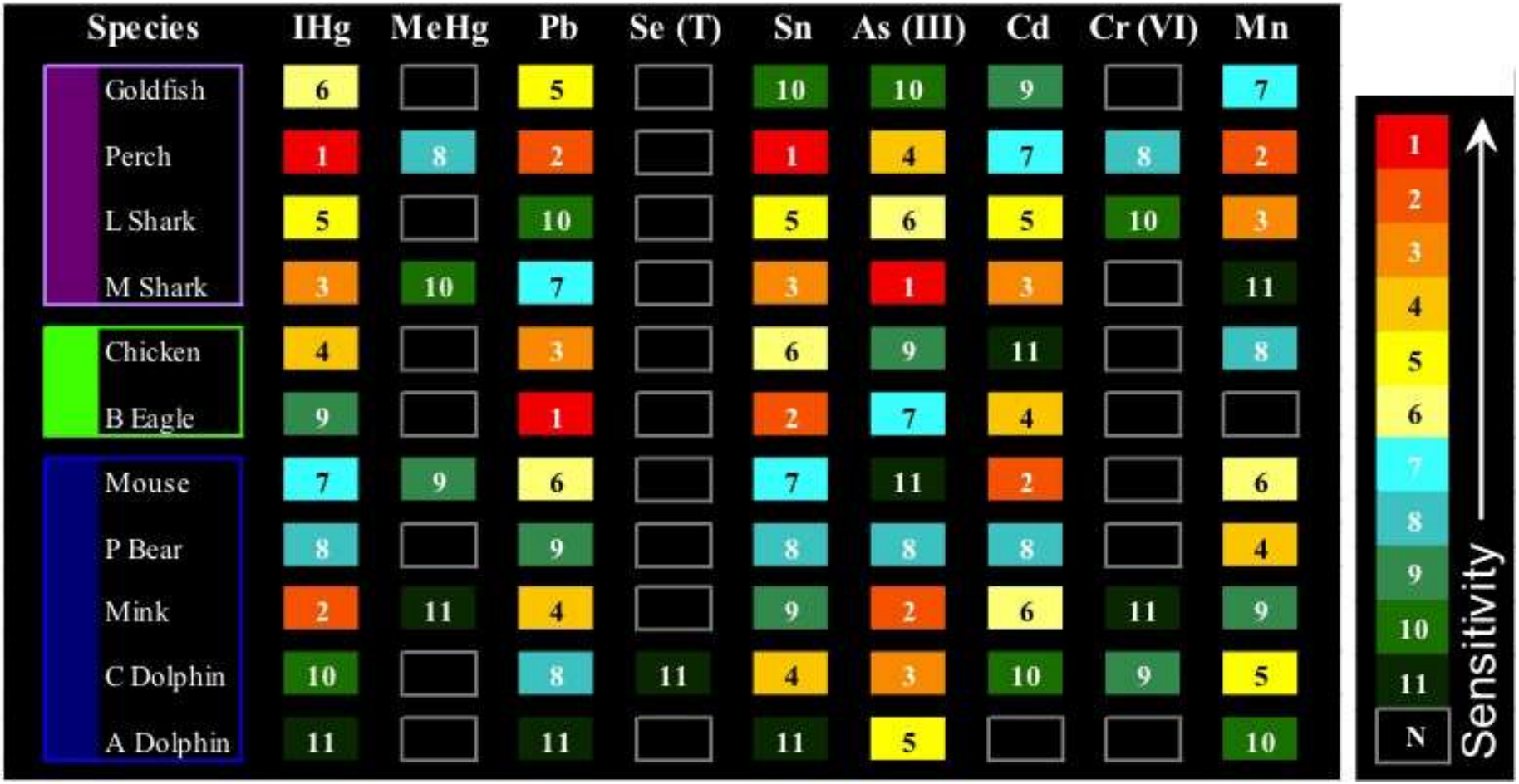
Pulp and Paper Mill Effluents Contain Neuroactive Substances That Potentially Disrupt Neuroendocrine Control of Fish Reproduction

NILADRI BASU,[†] CHIEU ANH TA,[‡] ANDREW WAYE,[‡] JINQIN MAO,[‡] MARK HEWITT,[§] JOHN T. ARNASON,[‡] AND VANCE L. TRUDEAU*,[‡]



D) Integrated "Heat Maps": 20 species, 16 assays, 60 toxicants

[below: 11 species x 9 metals x 2 assays = 198 datapoints]



Moving ahead-EPA STAR







Humans
Fish
Mammals
Birds

Metals
Pesticides
Pharmaceuticals
Organics
Mixtures

Acetylcholine
Dopamine
Glutamate
GABA

20 species

80-100 chemicals

16 endpoints

~32000 datapoints

AOP = Adverse Outcome Pathways

Toxicant

Chemical Structure and Reactivity Profiles

Macro-Molecular Interactions

Receptor/Ligand Interaction

DNA Binding

Protein Oxidation

Cellular Responses

Gene Activation

Protein Production

Altered Signaling

Protein Depletion

Organ Responses

Altered Physiology

Disrupted Homeostasis

Altered Tissue Development or Function

Individual Responses

Lethality

Impaired Development

Impaired Reproduction

Cancer

Population Responses

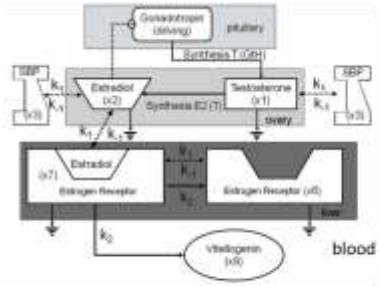
Structure

Recruitment

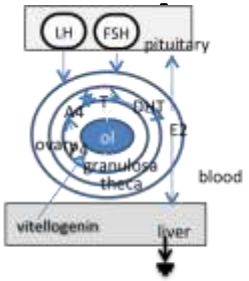
Extinction

Species	IHg	MeHg	Pb	Se (T)	Sn	As (III)	Cd	Cr (VI)	Mn
Goldfish	6		5		10	10	9		7
Perch	1	8	2		1	4	7	8	2
L Shark	5		10		5	6	5	10	3
M Shark	3	10	7		3	1	3		11
Chicken	4		3		6	9	11		8
B Eagle	9		1		2	7	4		
Mouse	7	9	6		7	11	2		6
P Bear	8		9		8	8	8		4
Mink	2	11	4		9	2	6	11	9
C Dolphin	10		8	11	4	3	10	9	5
A Dolphin	11		11		11	5			10

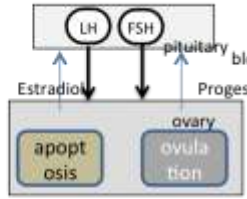
1 2 3 4 5 6 7 8 9 10 11 N — Sensitivity



A. Fish



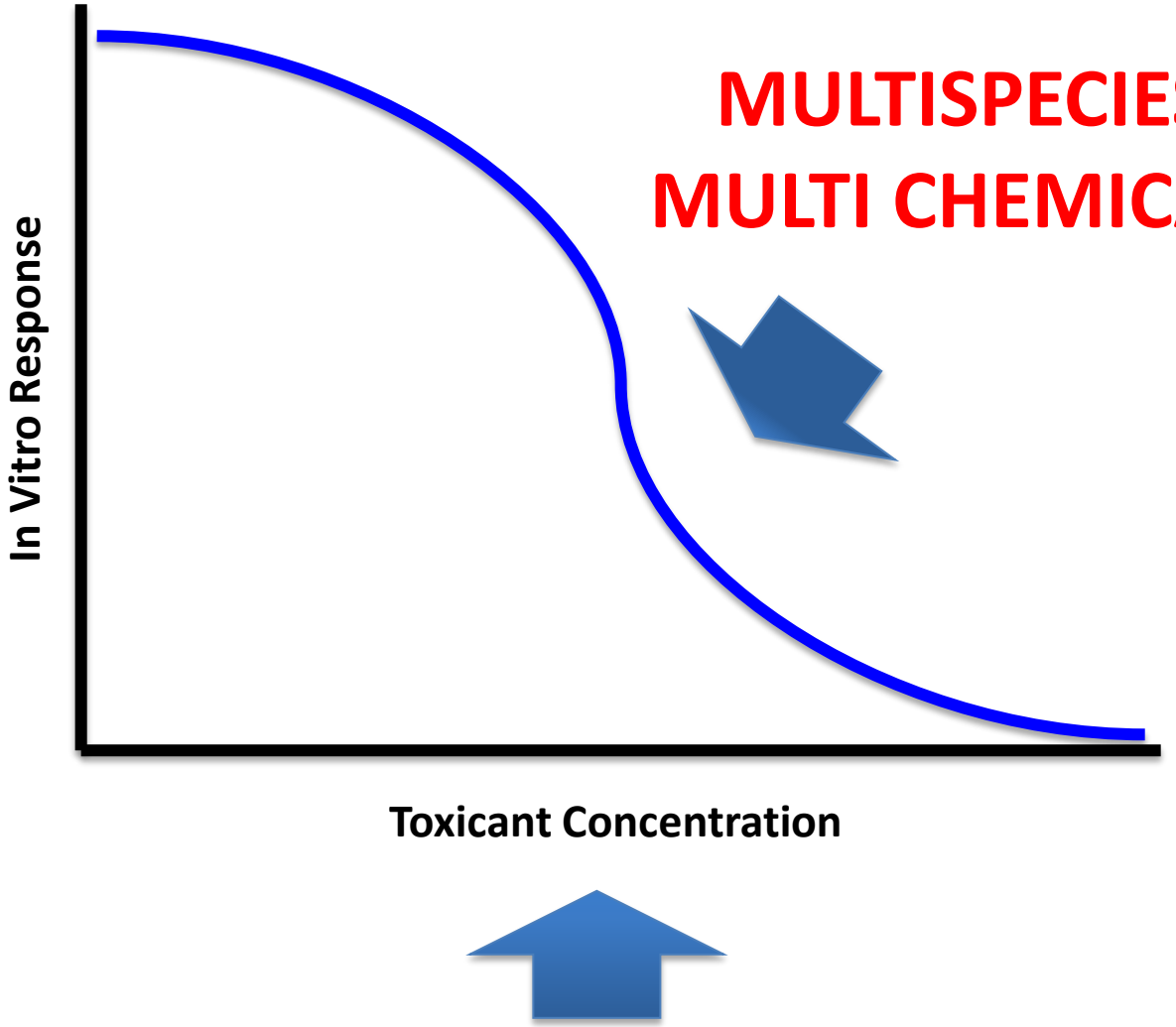
B. Bird



C. Mammal

Concluding Remark – Ultimate Goal

IN VIVO RESPONSES;
MODEL INTO AOPs;
PREDICT RISK VIA HTS



MULTISPECIES;
MULTI CHEMICALS

TISSUE RESIDUE VALUES; PRIORITY SUBSTANCES; ENVIRONMENTAL MIXTURES

Funding

-EPA-GLRI [EPAGLNPO-2010-TX-8-1223-395]

-EPA-STAR [R835170]

-Univ Mich School of Public Health

Questions?

sitemaker.umich.edu/ecotoxicology.lab

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